Ichthyosis Bullosa of Siemens—A Disease Involving Keratin 2e

W.H. Irwin McLean, Susan M. Morley, E. Birgitte Lane, Robin A.J. Eady,* W. Andrew D. Griffiths,* David G. Paige,† John I. Harper,† Caroline Higgins,‡ and Irene M. Leigh‡

CRC Cell Structure Research Group, Cancer Research Campaign Laboratories, Department of Anatomy & Physiology, Medical Sciences Institute, University of Dundee, Dundee; *St. John's Institute of Dermatology, United Medical and Dental Schools, St.Thomas's Hospital; †Dermatology Department, The Hospital for Sick Children; and ‡Experimental Dermatology Laboratories, Medical College of the Royal London Hospital, London, U.K.

Ichthyosis bullosa of Siemens (IBS) is a congenital bullous ichthyosis without erythroderma. In contrast to bullous congenital ichthyosiform erythroderma (BCIE), there is a relatively mild involvement of the skin and epidermolytic hyperkeratosis (EHK) is restricted to the upper suprabasal layers of the epidermis. Tonofilament aggregation was observed by EM in suprabasal cells from affected patients in the two families under study, indicative of a keratin abnormality. Keratin 2e is a differentiation specific type II keratin expressed suprabasally in the epidermis. Part of the K2e gene was amplified by polymerase chain reaction using genomic DNA from affected and unaffected individuals from two IBS families. Direct sequencing of polymerase chain reaction products revealed a point mutation in the highly conserved helix termination motif, producing the protein sequence change LLEGEE–LLEGKE. This mutation was found in all affected members of a five-generation kindred and also in a sporadic case in a second unrelated family. No mutation was seen in unaffected individuals. The mutation destroys a *MnlI* restriction site, which allowed exclusion of the mutation from a population of 50 unaffected unrelated individuals by restriction fragment analysis of K2e PCR products. This is the sixth keratin gene found to be involved in an inherited epidermal disorder. ***Keywords: epidermolytic hyperkeratosis (EHK)/intermediate filaments/keratin mutation/K2/epidermis/differentiation. J Invest Dermatol 103:277–281, 1994***

Ichthyosis bullosa of Siemens (IBS) (MIM number 146800 [1]) is a mild type of epidermolytic ichthyosis first described by Siemens in 1937 [2] as a separate disorder from bullous congenital ichthyosiform erythroderma of Brocq (BCIE, MIM number 113800 [1]). The phenotype of this disorder was further delineated more recently [3–5]. The disease is characterised by mild changes particularly on flexures, shins, and periumbilical region. Blistering occurs in response to mild physical trauma and results in superficial erosion or "moulting" of the outer skin. Erythroderma is not present. Keratin filament aggregates are seen by electron microscopy in the granular and upper spinous layers of the epidermis.

The cytoskeleton of epithelial cells is largely composed of keratin intermediate filaments. In epidermis, the basal cells express K5 (type II) and K14 (type I) predominantly. In suprabasal cells, synthesis of these proteins is replaced by differentiation-specific keratins K1 (type II) and K10 (type I) [6]. An additional type II keratin, K2e, is expressed in epidermis [7,8]. This is a protein of molecular weight 65.8 kDa encoded by a 2.6-kb mRNA species. Another keratin of similar molecular weight (K2p) has been shown to be of a different amino acid composition, is encoded by a different gene, and is expressed specifically in hard palate [9]. Recently, the K2e cDNA sequence was determined [8]. K2e is most similar to K1 in structure, consistent with its suprabasal expression pattern, which is reported to appear in the third or fourth cell layers of the epidermis, continuing outwards. It is expressed in epidermis from a number of body sites [8].

The role of keratins in maintaining the structural integrity of the epidermis has come to light in recent years with the finding that point mutations in K5 and K14 lead to various forms of epidermolysis bullosa simplex (EBS). In EBS, cytolysis occurs in the basal cell layer where K5 and K14 are specifically expressed. Similarly, mutations in K1 or K10 lead to suprabasal cytolysis and results in bullous congenital ichthyosiform erythroderma (BCIE), also called epidermolytic hyperkeratosis (EHK) (recently reviewed [10]). More recently, point mutations in the palm and sole specific K9 have been shown to be responsible for epidermolytic palmoplantar keratoderma (EPPK) [11]. Here we describe a mutation in K2e associated with IBS in two unrelated British families.

MATERIALS AND METHODS

Light and Electron Microscopy Biopsy samples of clinically affected skin from four members of family 1 and the single affected member of family 2 were processed for light and electron microscopy as described previously [12]. Briefly, the samples were fixed first in half-strength Karnovsky fixative, then in 1.3% aqueous osmium tetroxide and then dehydrated in ethanol and embedded in epoxy resin (Taab Laboratories Equipment Ltd., Aldermaston, Berkshire, UK). Semithin (1 μm) and ultrathin (~80 nm) sections were cut on a Reichert OMU4 ultramicrotome (Leica UK Ltd., Milton Keynes, UK). Semithin sections were stained with methylene blue and azure II (Richardson) for light microscopy. Ultrathin sections were stained with

Manuscript received April 21, 1994; revised May 25, 1994; accepted for publication June 10, 1994.

Reprint requests to: Dr. W.H.I. McLean, CRC Laboratories, Department of Anatomy and Physiology, Medical Sciences Institute, University of Dundee, Dundee DD1 4HN, U.K.

Abbreviations: BCIE, bullous congenital ichthyosiform erythroderma; IBS, ichthyosis bullosa of Siemens.

0022-202X/94/$07.00

lead citrate and uranyl acetate and viewed in a JEOL 100 CX transmission electron microscope, operating at 80 kV. Ultrathin sections of normal epidermis were prepared and examined for comparative purposes.

Polymerase Chain Reaction (PCR) A 1.5-kb fragment (approximately) of the K2e gene was amplified from genomic DNA using primers K2-5 (5′ GCA GTG TAA GAA TGT GCA AGA TG 3′, + strand) and K2-6 (5′ CAG TCA CAT TGC TGC TGA GG 3′, − strand). Primers were designed from the cDNA sequence [8] using the program PRIMER version 0.5 [13] and, due to the high degree of conservation between K2e and K1, primers were checked against a K1/K2e DNA alignment for K2e specificity. Due to the proximity of the helix termination sequence to the predicted position of intron VII, this intron is included in the K2-5/K2-6 fragment. Intron VII is about 1.25 kb in size. PCR was performed in 100-μl reaction volumes with standard buffer with 1 mM $MgCl_2$ and 10% dimethylsulfoxide and 1 U of Amplitaq polymerase (Perkin-Elmer-Cetus, CA). After an initial incubation at 94°C for 5 min, PCR was carried out for 30 cycles consisting of 94°C for 30 seconds, 55°C for 1 min, and 72°C for 2 min on an Omnigene Temperature Cycler (Hybaid, UK).

Cycle Sequencing PCR fragments were resolved on 1.5% low melting point agarose gels and excised bands purified using Gelase (Epicentre, USA). Approximately 50 fmol of purified template DNA was sequenced by the DS-Cycle Sequencing system (BRL, Bethesda, MD) using a [^{32}P] end-labeled internal primer, K2-7 (5′ GTT GAA TGA CCT GGA GGA GG 3′, + strand). Cycle sequencing was performed using the following program: 20 cycles consisting of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 min; followed by 10 cycles consisting of 94°C for 30 seconds, 72°C for 1 min. Sequencing ladders were resolved on standard 6% polyacrylamide denaturing gels.

Mutation Screening by Restriction Fragment Length Polymorphism (RFLP) The mutation found in both families destroys a recognition site for the restriction enzyme *Mnl*I. Due to the close proximity of intron VII, it was necessary to sequence part of this intron to design a primer for screening purposes. This was carried out by cycle sequencing as above with primer K2-7 (above). For screening, a 163-bp fragment containing the mutation was PCR amplified using primers K2-7 and K2-14 (5′ TTC CCA GTG CCC ACA CCT G 3′, − strand, intron VII). Primer K2-14 contains a 1-bp mismatch to remove an unwanted *Mnl*I site that would mask the mutated site in the assay. PCR was carried out in 25 μl volumes in microplates using the conditions described above except that primer K2-14 was end-labeled with [^{32}P]. The following PCR program was used: 30 cycles consisting of 94°C for 30 seconds, 55°C for 1 min, 72°C for 1 min. After PCR, 5 μl of 6 × *Mnl*I buffer containing 6 mM spermidine was added. Following incubation at 60°C for 10 min, samples were cooled and incubated for 4 h at 37°C with 1 U of *Mnl*I (New England Biolabs). An additional *Mnl*I site in the fragment is located 6 bp 5′ from the mutated site so that digests were denatured and resolved on 6% sequencing gels. Digestion of normal alleles gives rise to a 34-bp fragment. Digestion of the mutant allele gives rise to a 40-bp fragment. Affected heterozygotes produce 34- and 40-bp fragments.

RESULTS

IBS Is Clinically Distinct from BCIE The proband in family 1 is a 10-year-old girl **(Fig 1)** who was born with normal skin. She has never been erythrodermic. Blisters developed within a few months of age, mainly localized to the hands and legs, precipitated by physical trauma. From the age of 6 months, keratotic lichenification was noted over the extensor aspects of the elbows and knees, the wrists, and the periumbilical area. On the upper, outer aspects of the arms, superficial erosion of skin was seen, a phenomenon known as mauserung or moulting phenomenon. Nails, hair, and oral mucosa were normal. There was no palmoplantar keratoderma. Other members of the family are affected in five consecutive generations. The proband in family 2 has a phenotype indistinguishable from those affected in family 1. No other family members are affected.

Outer Suprabasal Cells in IBS Contain Abnormal Tonofilaments The microscopic findings were essentially the same in all biopsy samples. Light microscopy showed hyperkeratosis with poorly compacted cornified cells; slight or moderate acanthosis and papillomatosis; and a minimal inflammatory cell infiltrate in the superficial dermis. Epidermal cells from the mid-spinous layer up to and including the granular layer appeared enlarged with a pale edematous or vacuolated cytoplasm. Disintegration of the epidermis associated with cell lysis was not apparent. These altered cells could occur focally in groups rather than diffusely throughout the upper epidermis. Keratohyalin granules were often small and indistinct.

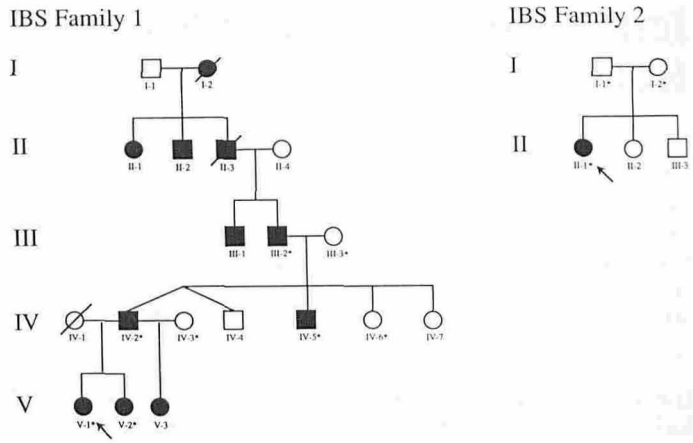


Figure 1. IBS is inherited in an autosomal dominant fashion. Pedigrees of IBS families under study. *Arrows,* probands in each family. Family 1 shows clear autosomal dominant Mendelian inheritance; family 2 is a sporadic case. Individuals are numbered by standard genetics notation; *, those which took part in this study.

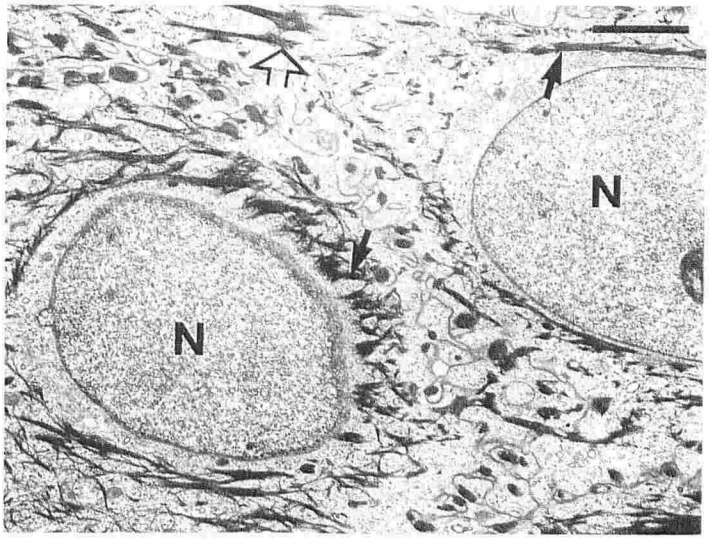
In some sections, the stratum corneum had become detached just above its normal junction with the granular layer.

Electron microscopy revealed a structurally normal basal and lowermost suprabasal layer. Bundling or clumping of the tonofilaments was evident in the mid-spinous layer and became more marked in the the upper spinous and lower granular layer **(Fig 2*a*)**. The filament clumps tended to be dispersed more in the cell periphery than around the nuclei; much of the cytoplasm around the cells edematous by light microscopy was filament free and contained polyribosomes, mitochondria and, in the granular layer, lamellar granules. Keratohyalin was associated with the clumps and also occurred as small round granules associated with polyribosomes **(Fig 2*a*)**. In normal spinous or granular epidermal keratinocytes the tonofilaments were often found in loose bundles **(Fig 2*b*)** but never formed the tight clumps as seen in IBS skin.

IBS Patients Are Heterozygous for a K2e Mutation A 1.5-kb fragment of the K2e gene was amplified from genomic DNA samples using primers K2-5 and K2-6. This fragment contains all of exon 7, which encodes part of the 2B domain and the helix termination peptide of K2e, intron VII, and a small portion of exon 8 encoding the V2 domain. 256 bp of this PCR fragment are coding sequence and so the deduced size of intron VII is about 1.25 kb, about twice the size of the equivalent intron in K1. However, the position of the intron was determined by direct sequencing of the PCR product and is completely conserved, in keeping with the other type II keratins [14]. Intron splice sites were found to conform to the reported consensus sequences [15].

Gel-purified PCR products derived from affected and unaffected individuals from IBS families 1 and 2 were directly sequenced using an internal primer K2-7, located in exon 7. In five unaffected individuals analyzed from families 1 and 2 and in four normal, unrelated individuals, the coding sequence was found to be identical to the published cDNA sequence, with no polymorphisms or silent mutations observed. However, all affected individuals analyzed in both IBS families (five in total) were found to be heterozygous for a G to A transition at nucleotide 1510 of the cDNA sequence ($G_{1510}A$) as shown in **Fig 3*a***. This produces a change of glutamic acid to lysine in codon 493 ($E_{493}K$) in the highly conserved LLEGEE helix termination motif and thus is likely to be highly detrimental to keratin filament assembly and/or functional integrity **(Fig 3*b*)**. This mutation is analogous to those reported in K5 causing EBS-DM [16] and

a



b

Figure 2. Suprabasal tonofilaments are abnormal in IBS. *a)* In IBS epidermis, tonofilaments *(small arrows)* appear normal in basal layer *(B)* and lowest suprabasal layer, but are replaced by abnormal clumps or amorphous bodies *(open arrow)* in the mid-spinous layer. Note that the clumps tend to be distributed in the cell periphery. *Bar,* 2 μm. *b)* Electron micrograph of normal keratinocytes in upper spinous layer. The tonofilaments in these cells, in contrast to IBS skin *(a),* form loose bundles *(arrows)* around the nuclei (N), extending peripherally to associate with desmosomes *(open arrow). Bar,* 2 μm.

in K1 causing BCIE [17] thus demonstrating once again the sensitivity of this motif to pathologic mutation in keratin molecules.

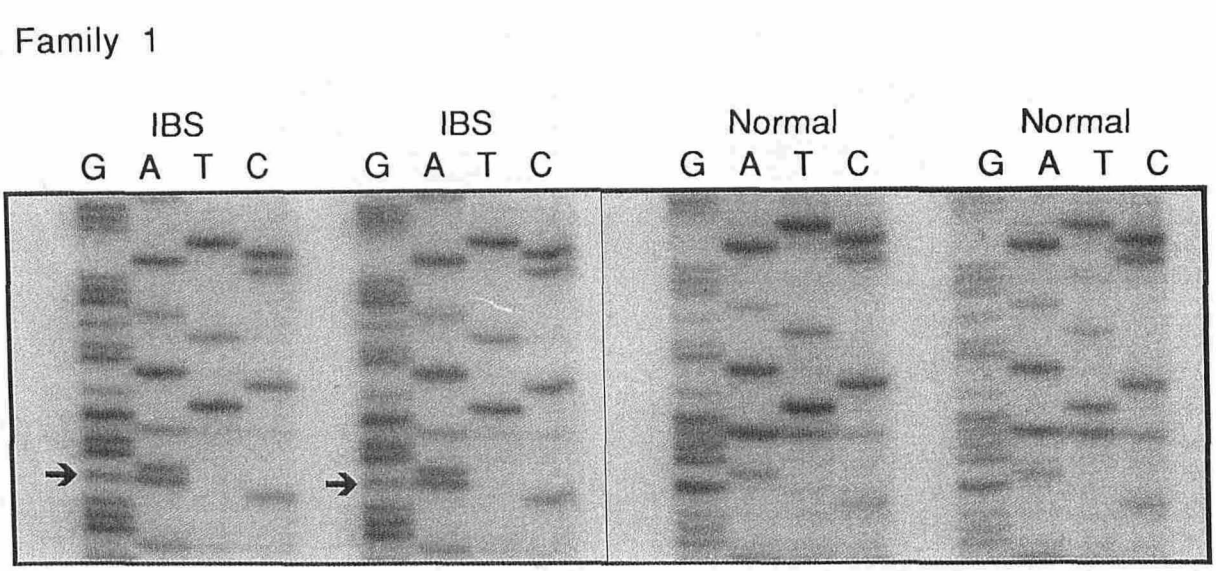
The mutation produces a *Mnl*I restriction fragment length polymorphism that was used to design a PCR-based screening test. Digestion of [^{32}P]-labeled PCR products amplified from normal individuals using primers K2-7 and K2-14 results in a 34-bp band. All affected members of both families were found to have an additional 40-bp band **(Fig 4)**. This test was used to exclude the mutation from 50 normal unrelated control samples indicating that this mutation is not a common polymorphism.

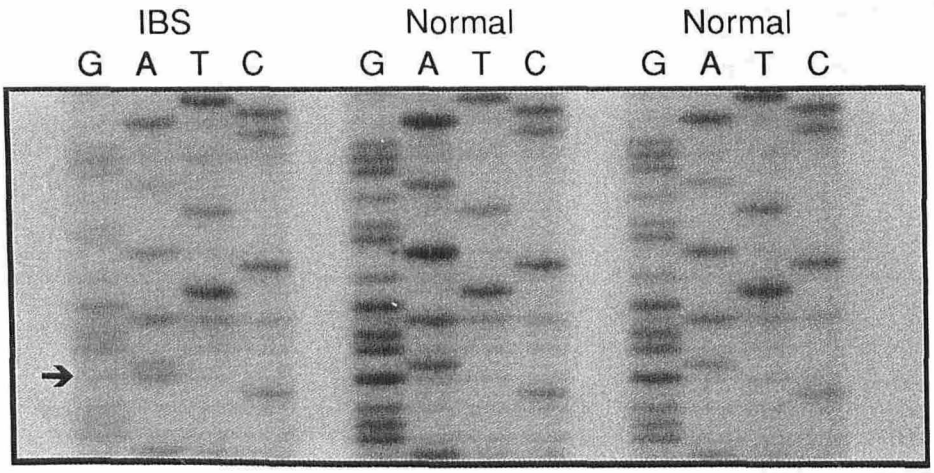
Members of family 2 were genotyped with a number of highly polymorphic markers (data not shown). Normal Mendelian inheritance with no evidence of non-paternity was observed; therefore, it is likely that this sporadic case represents a new mutation.

DISCUSSION

The Observed K2e Mutation May Be a Common Cause of IBS Here we describe (with Kremer *et al* [18]) mutations in K2e that are associated with IBS, a pathologic condition affecting the outer layers of the epidermis. The base change observed in two unrelated incidences is consistent with a methylated CpG deamination mutation of the 5-methyl-cytosine on the antisense strand at position 1510. This results in a CG to CA transition in the sense strand. Methylated CpG sequences have a higher mutation rate than other dinucleotides, taking the obvious bias for deleterious mutations into account [19]. Here, IBS family 1 has affected members in five generations; however, the affected member of IBS family 2 represents a new mutation causing this disease, the parents having been shown to have a normal K2e sequence. Kremer *et al* [18] describe two further unrelated Dutch families with identical mutations. The occurrence of the same mutation in four apparently unrelated families including one sporadic case indicates that this is likely to be a common mutation causing IBS.

IBS Ultrastructural Changes Are Consistent with Phenotype At the electronmicroscopic level, IBS is a disorder characterized by filament aggregation in the upper suprabasal cells leading to





a

```
                                                    MnlI          MnlI
Normal   1486 ACC TAC CGC AAA CTG CTG GAG GGC GAG GAG 1515
          485  T   Y   R   K   L   L   E   G   E   E  494

                                                    MnlI
IBS      1486 ACC TAC CGC AAA CTG CTG GAG GGC AAG GAG 1515
          485  T   Y   R   K   L   L   E   G   K   E  494
```

b

Figure 3. DNA Sequencing for Families 1 and 2. *a)* IBS individuals are heterozygous for a G to A mutation in K2e. Excerpts from DNA sequencing gels showing K2e sequence derived from IBS affected and unaffected individuals in both families 1 and 2. IBS individuals are shown to be heterozygous for a G to A transition corresponding to nucleotide 1510 of the cDNA sequence *(arrows). b)* The IBS mutation in both families results in an E to K transition. DNA sequence and deduced protein sequence in the region of the reported mutation showing the loss of an *Mnl*I restriction site (GGAG) in the mutant allele. The LLEGEE motif is most highly conserved within the various intermediate filament proteins.

hyperkeratosis and shedding of the stratum corneum. There are a number of ultrastructural features that are distinct from BCIE. Firstly, frank lysis of epidermal cells is not a prominent feature. Secondly, tonofilament clumps were not seen in the lower epidermis and also tended to be distributed more peripherally in the cells. The clinically superficial erosions or "moulting" probably results from separation of the stratum corneum at a low level. This could be due to abnormal structure of horny cells as reported in BCIE [20].

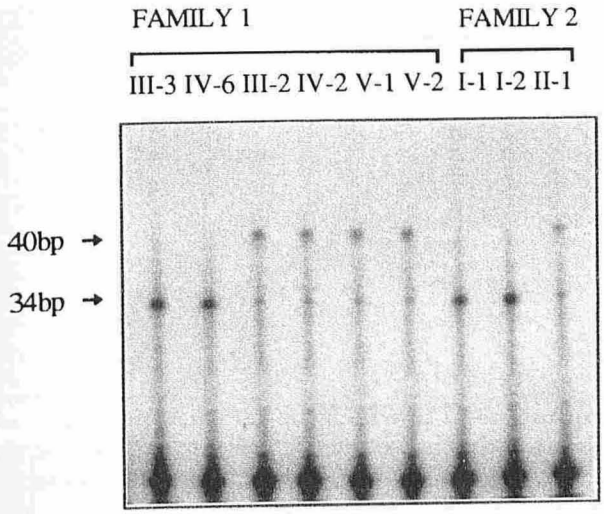


Figure 4. IBS individuals are heterozygous for a *MnlI* polymorphism. Restriction fragment analysis of *MnlI* digested PCR products derived from individuals in families 1 and 2. Normal allele gives rise to a 34-bp fragment. The mutant allele reseults in a 40-bp fragment. Affected heterozygotes produce both 34- and 40-bp fragments. Pedigree numbers correspond to **Fig 1.**

These ultrastructural differences between IBS and BCIE are in agreement with the differences in expression between K1/K10 and K2e suprabasally [8].

The observed ultrastructural changes result in the observed clinical phenotype of the disease—very superficial epidermolytic hyperkeratosis, with scaling confined to the outermost layers of the skin. The disease tends to preferentially affect certain body sites—the elbows and knees where the epidermis is subject to a lot of flexion and/or abrasion. Further work is required, using specific antibodies or *in situ* hybridization, to determine the expression of K2e at these different body sites.

The Observed IBS Mutations Cause Keratin Filament Aggregation IBS falls into a category of diseases where grossly abnormal filaments are seen by EM. The other examples where tonofilament clumping is evident are EBS-DM, BCIE, and EPPK. The unifying feature of all four of these diseases is the location of mutation in the keratin protein, within the highly conserved ends of the rod domain. In EBS-WC and EBS-K, the milder EBS variants, mutations in K5 and K14 are found in locations other than the helix initiation and termination peptides (reviewed in [10]). There is a body of evidence that indicates that the rod end sequences are important in production of normal 10-nm filaments—transfection experiments [21,22], transgenics [23], and now disease causing mutations in 6 keratins [10].

The question arises as to why these motifs are so sensitive to sequence alteration. Cross-linking studies have been interpreted as suggesting that there is a 1A/2B overlap involved in higher-order assembly of intermediate filaments [24]. The manner of such an interaction is as yet unknown although studies using synthetic peptides of desmin rod ends has shown that these tend to self-aggregate into filamentous structures that contain beta-sheet conformation [25]. The region where mutations produce filament aggregation is slowly being defined by the discovery of more keratin mutations. If the rod ends are really involved in overlap interactions between dimers or tetramers, these interactions would explain why mutations in these parts of the protein are severe in their effects. However, the evidence for this is largely circumstantial at present and awaits direct experimental proof.

Thanks to John McGrath, St. Johns Insitute of Dermatology and Adrian Heagarty, Department of Dermatology, North Staffordshire Royal Infirmary, Stoke-on-Trent, UK for clinical assistance; Trish Dopping-Hepenstal and Ian Palmer for technical help; and Hannie Kremer, Department of Human Genetics, University Hospital Nijmegen for sharing data and for helpful discussions. Thanks to Dot Mechan and David Baty, Genetics Department, Ninewells Hospital, Dundee for DNA processing. This work was supported by grants from The Wellcome Trust (037444/A/93/Z: EBL, RAJE, and IML), Cancer Research Campaign (SP2060, EBL), Muirhead Trust (RAJE), and Dystrophic Epidermolysis Bullosa Research Association (EBL).

REFERENCES

1. McKusick VA: *OMIM (On-line Mendelian Inheritance in Man).* Johns Hopkins University, Baltimore
2. Siemens HW: Dichtung und Wahrheit über die die "Ichthyosis bullosa", mit Bemerkungen zur Systematik der Epidermolysen. *Arch Dermatol Syph (Berl)* 175:590–608, 1937
3. Traupe H, Kolde G, Hamm H, Happle R: Ichthyosis bullosa of Seimens: a unique type of epidermolytic hyperkeratosis. *J Am Acad Dermatol* 14:1000–1005, 1986
4. Traupe H: *The ichthyoses. A guide to clinical diagnosis, genetic counselling, and therapy.* Springer-Verlag, Berlin, 1989
5. Steijlen PM, Perret CM, Schuurmans Stekhoven JH, Ruiter DJ, Happle R: Ichthyosis bullosa of Siemens: further delineation of phenotype. *Arch Dermatol Res* 282:1–5, 1990
6. Lane EB: In: *Keratins.* Royce PM, Steinmann B (eds.). *Connective Tissue and its Heritable Disorders. Molecular, Genetic and Medical Aspects.* Wiley-Liss Inc., New York, 1993
7. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R: The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 31:11–24, 1982
8. Collin C, Moll R, Kubicka S, Ouhayoun J-P, Franke WW: Characterization of human cytokeratin 2, an epidermal cytoskeleton protein synthesized late during differentiation. *Exp Cell Res* 202:132–141, 1992
9. Collin C, Ouhayoun J-P, Grund C, Franke WW: Suprabasal marker proteins distinguishing keratinizing squamous epithelia: cytokeratin 2 polypeptides of oral masticatory epithelium and epidermis are different. *Differentiation* 51:137–148, 1992
10. Lane EB: Keratin Diseases. *Curr Opinion Genet Devel* 4:412–418, 1994
11. Reis A, Hennies H-C, Langbein L, Digweed M, Mischke D, Drechsler M, Schröck E, Royker-Pokora B, Franke WW, Sperling K, Küster W: Keratin 9 gene mutations in epidermolytic palmoplantar keratoderma (EPPK). *Nature Genet* 6:174–179, 1994
12. Ishida-Yamamoto A, McGrath JA, Judge MR, Leigh IM, Lane EB, Eady RAJ: Selective involvement of keratins K1 and K10 in the cytoskeletal abnormality of epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma). *J Invest Dermatol* 99:19–26, 1992
13. Lincoln SE, Daly MJ, Lander ES: PRIMER: a computer program for automatically selecting PCR primers. MIT Center for Genome Research, Cambridge, Massachusetts
14. Dodemont H, Riemer D, Weber K: Structure of an invertebrate gene encoding cytoplasmic intermediate filament (IF) proteins: implications for the origin and the diversification of IF proteins. *EMBO J* 9:4083–4094, 1990
15. Padgett RA, Grabowski PJ, Konarsa MM, Seiler S, Sharp PA: Splicing of messenger RNA precursors. *Ann Rev Biochem* 55:1119–1150, 1986
16. Lane EB, Rugg EL, Navsaria H, Leigh IM, Heagerty AHM, Ishida-Yamamoto A, Eady RAJ: A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. *Nature* 356:244–246, 1992
17. Rothnagel JA, Dominey AM, Dempsey LD, Longley MA, Greenhalg DA, Gagne TA, Huber M, Frenk E, Hohl D, Roop DR: Mutations in the rod domains of keratins 1 and 10 in epidermolytic hyperkeratosis. *Science* 257:1128–1130, 1992
18. Kremer H, Steijlen PM, *et al*: Ichthyosis bullosa of Siemens is caused by mutations in the keratin 2e gene. *J Invest Dermatol* 103:286–289, 1993 (this issue)
19. Cooper DN, Krawczak M: *Human Gene Mutation.* Bios Scientific Publishers, Oxford, 1993
20. Ishida-Yamamoto A, Eady RAJ, Underwood RA, Dale BA, Holbrook KA: Filaggrin expression in epidermolytic ichthyosis (epidermolytic hyperkeratosis). *Br J Dermatol* (in press)
21. Albers K, Fuchs E: The expression of mutant epidermal keratin cDNAs transfected in simple epithelial and squamous cell carcinoma lines. *J Cell Biol* 105:791–806, 1987
22. Albers K, Fuchs E: Expression of mutant keratin cDNAs in epithelial cells reveals possible mechanisms for initiation and assembly of intermediate filaments. *J Cell Biol* 108:1477–1493, 1989
23. Vassar R, Coulombe PA, Degenstein L, Albers K, Fuchs E: Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin disease. *Cell* 64:365–380, 1991
24. Steinert PM, Marekov LN, Fraser RDB, Parry DAD: Keratin intermediate filament structure: crosslinking studies yield quantitative information on molecular dimensions and mechanism of assembly. *J Mol Biol* 230:436–452, 1993
25. Geisler N, Heimburg T, Schünemann J, Weber K: Peptides from the conserved ends of the rod domain of desmin disassemble intermediate filaments and reveal unexpected structural features: a circular dichroism, fourier transfrom infrared, and electron microscopic study. *J Struct Biol* 110:205–214, 1993